IB. AMENDMENTS TO THE CLAIMS

Please enter the amendments to claims 1, 5, 9, 11, and 15, as shown below.

Please enter new claims 61-90, as shown below.

1. (Currently amended) A method for synthesizing isopentenyl pyrophosphate (IPP) via a mevalonate pathway in a host microorganism, wherein the method comprises:

culturing a transformed host microorganism in a suitable medium, the transformed host microorganism comprising one or more nucleic acids heterologous to the host microorganism, wherein the one or more nucleic acids comprises nucleotide sequences encoding two or more enzymes in the mevalonate pathway, and wherein the host microorganism is a prokaryote that does not normally synthesize IPP through the mevalonate pathway; wherein the mevalonate pathway comprises:

(a) condensing two molecules of acetyl-CoA to acetoacetyl-CoA;

(b) condensing acetoacetyl-CoA with acetyl-CoA to form HMG-CoA;

(c) converting HMG-CoA to mevalonate;

(d) phosphorylating mevalonate to mevalonate 5-phosphate;

(e) converting mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and

(f) converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate,

said culturing providing for production of the <u>two or more</u> enzymes,

wherein said production of said two or more enzymes results in production of IPP.

2. (Previously presented) The method of claim 1, wherein the one or more heterologous nucleic acids is integrated into the chromosome of the host microorganism.

3. (Previously presented) The method of claim 1, wherein the one or more heterologous nucleic acids is contained in at least one extrachromosomal expression vector.

4. (Previously presented) The method of claim 3, wherein the one or more heterologous nucleic acids is present in a single expression vector.

5. (Currently amended) A ~~The~~ method ~~of claim 4,~~ for synthesizing isopentenyl pyrophosphate (IPP) via a mevalonate pathway in a host microorganism, wherein the method comprises:

culturing a transformed host prokaryote microorganism that does not normally synthesize IPP through the mevalonate pathway in a suitable medium, the transformed host microorganism comprising ~~wherein the~~ a single extrachromosomal expression vector heterologous to the host microorganism that comprises the nucleotide sequence set forth in SEQ ID NO 7 or a fragment thereof encoding the enzymes in a mevalonate pathway; wherein the mevalonate pathway comprises:

(a) condensing two molecules of acetyl-CoA to acetoacetyl-CoA;

(b) condensing acetoacetyl-CoA with acetyl-CoA to form HMG-CoA;

(c) converting HMG-CoA to mevalonate;

(d) phosphorylating mevalonate to mevalonate 5-phosphate;

(e) converting mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and

(f) converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate,

said culturing providing for production of the enzymes, wherein said production of said enzymes results in production of IPP.

6. (Previously presented) The method of claim 3, wherein each of the one or more heterologous nucleic acids is contained within a separate expression vector.

7. (Previously presented) The method of claim 3, wherein at least two of the one or more heterologous nucleic acids are contained in a single expression vector.

8. (Previously presented) The method of claim 3, wherein the one or more heterologous nucleic acids is contained in two expression vectors.

9. (Currently amended) A ~~The~~ method ~~of claim 8,~~ for synthesizing isopentenyl pyrophosphate (IPP) via a mevalonate pathway in a host microorganism, wherein the method comprises:

culturing a transformed host prokaryote microorganism that does not normally synthesize IPP through the mevalonate pathway in a suitable medium, the transformed host microorganism comprising two extrachromosomal expression vectors, wherein the first expression vector comprises the nucleotide sequence set forth in SEQ ID NO 8, and the second expression vector comprises the nucleotide sequence set forth in SEQ ID NO 9 or a fragment thereof, which sequences from the two vectors are heterologous

to the host microorganism and encode the enzymes in a mevalonate pathway; wherein the mevalonate pathway comprises:

(a) condensing two molecules of acetyl-CoA to acetoacetyl-CoA;

(b) condensing acetoacetyl-CoA with acetyl-CoA to form HMG-CoA;

(c) converting HMG-CoA to mevalonate;

(d) phosphorylating mevalonate to mevalonate 5-phosphate;

(e) converting mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and

(f) converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate,

said culturing providing for production of the enzymes, wherein said production of said enzymes results in production of IPP.

10. (Previously presented) A method for synthesizing isopentenyl pyrophosphate (IPP) via a mevalonate pathway in a host microorganism, the method comprising:

culturing a transformed host microorganism in a suitable medium, the transformed host microorganism comprising one or more nucleic acids heterologous to the host microorganism, wherein the host microorganism is a prokaryote that does not normally synthesize IPP through the mevalonate pathway, wherein the one or more nucleic acids comprises nucleotide sequences encoding two or more enzymes selected from:

a) an enzyme capable of condensing two molecules of acetyl-CoA to acetoacetyl-CoA, wherein said enzyme is from *Ralstonia*, *Saccharomyces*, or *Escherichia coli*;

b) an enzyme capable of condensing acetoacetyl-CoA with acetyl-CoA to form HMG-CoA, wherein said enzyme is from *Blattella or Saccharomyces*;

c) an enzyme capable of converting HMG-CoA to mevalonate, wherein said enzyme is from *Sulfolobus*, *Haloferax*, or *Saccharomyces*;

d) a *Saccharomyces* enzyme capable of phosphorylating mevalonate to mevalonate 5-phosphate ;

e) a *Saccharomyces* enzyme capable of converting mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and

f) a *Saccharomyces* enzyme capable of converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate,

said culturing providing for production of the enzymes, wherein said production of said two or more enzymes results in production of IPP.

11. (Currently amended) A ~~The~~ method ~~of claim 10,~~ for synthesizing isopentenyl pyrophosphate (IPP) via a mevalonate pathway in a host microorganism, the method comprising:

culturing a transformed host microorganism in a suitable medium, the transformed host microorganism comprising one or more nucleic acids heterologous to the host microorganism, wherein the host microorganism is a prokaryote that does not normally synthesize IPP through the mevalonate pathway, wherein the one or more heterologous nucleic acids comprises nucleotide sequences encoding two or more enzymes selected from:

a) an enzyme capable of condensing two molecules of acetyl-CoA to acetoacetyl-CoA encoded by the nucleotide sequence of SEQ ID NO 1;

b) an enzyme capable of condensing acetoacetyl-CoA with acetyl-CoA to form HMG-CoA, encoded by the nucleotide sequence of SEQ ID NO 2;

c) an enzyme capable of converting HMG-CoA to mevalonate encoded by the nucleotide sequence of SEQ ID NO 3;

d) a *Saccharomyces* enzyme capable of phosphorylating mevalonate to mevalonate 5-phosphate encoded by the nucleotide sequence of SEQ ID NO 4;

e) a *Saccharomyces* enzyme capable of converting mevalonate 5-phosphate to mevalonate 5-pyrophosphate encoded by the nucleotide sequence of SEQ ID NO 5; and

f) a *Saccharomyces* enzyme capable of converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate encoded by the nucleotide sequence of SEQ ID NO 6,

said culturing providing for production of the enzymes, wherein said production of said two or more enzymes results in production of IPP.

12. (Previously presented) The method of claim 1, further comprising recovering the isopentenyl pyrophosphate from the transformed host microorganism.

13. (Previously presented) The method of claim 1, wherein the method further comprises reacting isopentenyl pyrophosphate with dimethylallyl pyrophosphate or a polyprenyl pyrophosphate in the presence of at least one enzyme to provide a polyprenyl pyrophosphate isoprenoid precursor.

14. (Previously presented) The method of claim 13, wherein the one or more heterologous nucleic acids further comprises:

g) a DNA fragment coding for an enzyme capable of converting isopentenyl pyrophosphate to dimethylallyl pyrophosphate.

15. (Currently amended) The method of claim 1, wherein the isopentenyl pyrophosphate is further modified enzymatically by the action of isopentenyl pyrophosphate isomerase and one or more polyprenyl pyrophosphate synthases to provide an isoprenoid selected from the group consisting of a monoterpene, sesquiterpene, diterpene, sesterterpene, triterpene, tetraterpene, and a steroid.

16. (Original) The method of claim 15, wherein the isoprenoid is a monoterpene.

17. (Original) The method of claim 16, wherein the monoterpene is selected from the group consisting of limonene, citranellol, and geraniol.

18. (Original) The method of claim 15, wherein the isoprenoid is a sesquiterpene.

19. (Original) The method of claim 18, wherein the sesquiterpene is selected from the group consisting of periplanone B, artemisinin, ginkgolide B, forskolin, and farnesol.

20. (Previously presented) The method of claim 15, wherein the isoprenoid is a diterpene.

21. (Original) The method of claim 20, wherein the diterpene is selected from the group consisting of casbene and paclitaxel.

22. (Canceled)

23. (Previously presented) The method of claim 1, wherein the prokaryote is *Escherichia coli.*

24.-60. (Canceled)

61. (New) The method of claim 5, wherein the prokaryote is *Escherichia coli.*

62. (New) The method of claim 8, wherein the prokaryote is *Escherichia coli.*

63. (New) The method of claim 9, wherein the prokaryote is *Escherichia coli.*

64. (New) The method of claim 10, wherein the prokaryote is *Escherichia coli.*

65. (New) The method of claim 11, wherein the prokaryote is *Escherichia coli.*

66. (New) The method of claim 15, wherein the prokaryote is *Escherichia coli.*

67. (New) The method of claim 64, wherein the enzyme capable of condensing two molecules of acetyl-CoA to acetoacetyl-CoA is an *E. coli* enzyme.

68. (New) The method of claim 64, wherein the enzyme capable of condensing acetyl-CoA with acetoacetyl-CoA is a *Saccharomyces* enzyme.

69. (New) A method for synthesizing isopentenyl pyrophosphate (IPP) via a mevalonate pathway in a host microorganism, wherein the method comprises:

culturing a transformed host microorganism in a suitable medium, the transformed host microorganism comprising at least two operons heterologous to the host microorganism, wherein each of said two operons comprises nucleotide sequences encoding enzymes in the mevalonate pathway, and wherein the host microorganism is a prokaryote that does not normally synthesize IPP through the mevalonate pathway; wherein the mevalonate pathway comprises:

(a) condensing two molecules of acetyl-CoA to acetoacetyl-CoA;

(b) condensing acetoacetyl-CoA with acetyl-CoA to form HMG-CoA;

(c) converting HMG-CoA to mevalonate;

(d) phosphorylating mevalonate to mevalonate 5-phosphate;

(e) converting mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and

(f) converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate,

said culturing providing for production of the enzymes,

wherein said production of said two or more enzymes results in production of IPP.

70. (New) The method of claim 69, wherein said at least two operons are contained in a single extrachromosomal expression vector.

71. (New) The method of claim 69, wherein at least one of said at least two operons is contained in a different extrachromosomal expression vector from another of said at least two operons.

72. (New) The method of claim 69, wherein at least one of said at least two operons is integrated into a chromosome of said transformed host microorganism.

73. (New) The method of claim 69, wherein said transformed host microorganism also comprises a heterologous nucleic acid comprising a nucleotide sequence encoding an enzyme that converts IPP to dimethylallyl pyrophosphate, and the method further comprises reacting the IPP with dimethylallyl pyrophosphate and a polyprenyl pyrophosphate synthase to provide a polyprenyl pyrophosphate isoprenoid precursor.

74. (New) The method of claim 69, wherein said transformed host microorganism is *E. coli*.

75. (New) The method of claim 70, wherein said transformed host microorganism is *E. coli*.

76. (New) The method of claim 71, wherein said transformed host microorganism is *E. coli*.

77. (New) The method of claim 72, wherein said transformed host microorganism is *E. coli*.

78. (New) The method of claim 74, wherein said *E. coli.* also produces IPP by a DXP pathway.

79. (New) The method of claim 74, wherein

a) said enzyme capable of condensing two molecules of acetyl-CoA to acetoacetyl-CoA is from *Ralstonia*, *Saccharomyces*, or *Escherichia coli*;

b) said enzyme capable of condensing acetoacetyl-CoA with acetyl-CoA to form HMG-CoA is from *Blattella or Saccharomyces*;

c) said enzyme capable of converting HMG-CoA to mevalonate is from *Sulfolobus*, *Haloferax*, or *Saccharomyces*; and

d) said enzymes capable of phosphorylating mevalonate to mevalonate 5-phosphate, capable of converting mevalonate 5-phosphate to mevalonate 5-pyrophosphate, and capable of converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate, are from *Saccharomyces*.

80. (New) The method of claim 74, wherein each of said at least two operons comprises a heterologous nucleic acid selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO 1;

b) the nucleotide sequence set forth in SEQ ID NO 2;

c) the nucleotide sequence set forth in SEQ ID NO 3;

d) the nucleotide sequence set forth in SEQ ID NO 4;

e) the nucleotide sequence set forth in SEQ ID NO 5; and

f) the nucleotide sequence set forth in SEQ ID NO 6.

81. (New) The method of claim 76, wherein the first vector contains the nucleotide sequence set forth in SEQ ID NO:8 and the second vector contains the nucleotide sequence set forth in SEQ ID NO. 9.

82. (New) The method of claim 79, wherein the enzyme capable of condensing two molecules of acetyl-CoA to form acetoacetyl-CoA is an *E. coli* enzyme.

83. (New) The method of claim 79, wherein the enzyme capable of condensing acetyl-CoA to acetoacetyl-CoA to form HMG-CoA is a *Saccharomyces* enzyme.

84. (New) The method of claim 1, wherein the two or more enzymes are from at least two distinct organisms.

85. (New) The method of claim 1, wherein at least one of the two or more enzymes is from an organism other than *Saccharomyces cerevisiae*.

86. (New) The method of claim1, wherein the production of IPP is evidenced by an overproduction of an isoprenoid in the transformed host microorganism as compared to a corresponding non-transformed microorganism.

87. (New) The method of claim 86, wherein the transformed host microorganism overproduces an isoprenoid by at least about 5 fold.

88. (New) The method of claim 1, wherein the one or more nucleic acids comprises nucleotide sequences encoding three enzymes in the mevalonate pathway.

89. (New) The method of claim 1, wherein the one or more nucleic acids comprises nucleotide sequences encoding four enzymes in the mevalonate pathway.

90. (New) The method of claim 1, wherein the one or more nucleic acids comprises nucleotide sequences encoding six enzymes in the mevalonate pathway.